The evidence for a role of B cells in multiple sclerosis

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ABSTRACT

Understanding the pathogenesis of complex immunologic disorders such as multiple sclerosis (MS) is challenging. Abnormalities in many different cell types are observed in the immune system and CNS of patients with MS and the identification of the primary and secondary events is difficult. Recent studies suggest that the model of MS as a disorder mediated only by T cells is overly simplistic and propose an important role for B cells in the propagation of the disease. B-cell activation in the form of oligoclonal bands (OCB) production is the most consistent immunologic finding in patients with MS. Notably, markers of B-cell activation within the CSF of patients with MS predict conversion from clinically isolated syndrome to clinically definite MS and correlate with MRI activity, onset of relapses, and disability progression. In addition, the main genetic risk factor in MS is associated with OCB production, and environmental agents associated with MS susceptibility (vitamin D and the Epstein-Barr virus) influence B-cell proliferation and function. Finally, the only cell-specific treatments that are effective in patients with MS are monoclonal antibodies targeting the B-cell antigen CD20, suggesting a potentially causative role for B cells. Based on current evidence there is no longer doubt that B cells are relevant to the etiology and pathogenesis of MS. Elucidating the role of B cells in MS will be a fruitful strategy for disease prevention and treatment. ***Neurology*® 2012;78:823–832**

GLOSSARY

CDMS = clinically definite multiple sclerosis; **ChIP-seq** = chromatin immunoprecipitation followed by massively parallel DNA sequencing; **CIS** = clinically isolated syndrome; **EAE** = experimental autoimmune encephalomyelitis; **EBNA** = Epstein-Barr nuclear antigen; **EBV** = Epstein-Barr virus; **GC** = germinal center; **IL** = interleukin; **IM** = infectious mononucleosis; **LCL** = lymphoblastoid cell line; **LMP** = latent membrane protein; **MBP** = myelin basic protein; **MHC** = major histocompatibility complex; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **OCB** = oligoclonal bands; **PLP** = proteolipid protein; **PPMS** = primary progressive multiple sclerosis; **RRMS** = relapsing-remitting multiple sclerosis; **SPMS** = secondary progressive multiple sclerosis; **Th** = T helper cells; **VDR** = vitamin D receptor; **VDRE** = vitamin D responsive element.

Multiple sclerosis (MS) is a debilitating disease of the CNS characterized by myelin loss, axonal pathology, and progressive neurologic dysfunction.[1] Genetic, epidemiologic, and pathologic studies support the hypothesis that the neurologic manifestations of the disease arise from immune-mediated demyelination, which impairs neuronal transmission and results in axonal degeneration.[2,3] However, understanding the factors driving this abnormal immune response is limited.

T cells and in particular CD4+ T helper cells (Th) have until recently been considered the primary immune drivers in MS. Evidence supporting this hypothesis includes that the main MS genetic risk factor resides in the major histocompatibility complex (MHC) class II region, which plays a central role in the development of T-cell central tolerance[4,5] and that the animal model of MS, experimental autoimmune encephalomyelitis (EAE), can be adoptively transferred to mice by the injection of encephalitogenic myelin-specific Th cells.[6]

Supplemental data at www.neurology.org

Supplemental Data

From the Wellcome Trust Centre for Human Genetics (G.D., J.M.M., S.V.R.), University of Oxford, Headington, Oxford; Nuffield Department of Clinical Neurosciences (Clinical Neurology) (G.D., J.M.M., S.V.R.), University of Oxford, John Radcliffe Hospital, Oxford, UK; Institute of Clinical Neurosciences (M.H.B.), Royal Prince Alfred Hospital, Sydney; Brain and Mind Research Institute (M.H.B.), University of Sydney, Sydney, Australia; and Blizard Institute (G.G., S.V.R.), Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, UK.

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However, the notion of MS being primarily a Th-cell disease has been reassessed, and the role of CD8+ T cells, B cells, and innate immunity emphasized. We review here recent evidence that B cells are important in driving the pathologic immune response in MS.

LIMITATIONS OF THE CD4+ MODEL Several features of MS plaque pathology cast doubt on the view that MS is a purely Th-cell–directed process. Lymphocytes may be absent in early MS lesions with extensive oligodendrocyte loss and demyelination.[7,8] Conversely, inflammatory cuffs are often present in otherwise normal-appearing white matter contiguous with or distant from active MS lesions and unlike the lesions of EAE, CD4+ T cells are outnumbered by MHC class I–restricted, clonally expanded CD8+ cells in active MS lesions.[9,10]

The necessity to re-evaluate the role of different cell types in MS was also recently suggested when a phase II clinical trial testing the effect of Ustekinumab, an antibody directed against the common subunit of interleukin (IL)-12 and IL-23, relevant for Th1 and Th17 cell differentiation respectively, reported no benefit in relapsing-remitting MS (RRMS).[11] Previously, an antibody directed against CD4, which successfully depleted CD4+ T cells, was also found to have no effect on disease activity or course in patients with MS.[12] In clear contrast, analogous antibodies prevented the development of EAE.[13,14] These trials demonstrate why, although animal models represent useful and essential tools in modern research, one needs to be aware of their distance from the human disease.[15] This is especially true in complex immune disorders such as MS, in which primary causal and secondary events are difficult to separate.

OLIGOCLONAL BANDS: THE HALLMARK OF MS The most consistent immunologic finding in patients with MS is the presence of oligoclonal bands (OCB) in the CSF. OCB arise from the intrathecal synthesis of clonal IgG and are present in more than 95% of patients with MS.[16] Clonally expanded B cells are also located in brain parenchyma and are responsible for the production of OCB.[17–19] Plasma cells are observed in large numbers in the perivascular spaces within subacute and chronic MS plaques, and it is likely that antigen processing and antibody synthesis takes place at these sites.[8,20] A lymph node sinus-like organization of the perivascular spaces is seen within plaques of chronic patients with MS, with lymphocytes and resident macrophages located within, and plasma cells located outside, smooth-walled channels.[20] Plasma cells are also present in smaller numbers in parenchymal plaque and periplaque white matter tissue, and the leptomeninges. This indicates a targeted B-cell response consequent to antigenic stimulation within the CNS.

The pathogenicity of these antibodies remains controversial. IgG deposition and complement-mediated demyelination are predominant features of brain lesions in patients with established disease.[21] However, in newly forming lesions, oligodendrocyte apoptosis is a striking feature that does not appear to be triggered by immunoglobulin deposition, T cells, or macrophages.[7,8] Additionally, the ability of these intrathecal antibodies to react against myelin antigens is unclear.[22] For example, a recent study has shown that recombinant antibodies generated from clonally expanded plasma cell and B-cell clones can react against MS brain tissue.[23] However, in a larger screen of more than 50 recombinant antibodies derived from the CSF of patients with MS, nearly all did not bind myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP) (the main candidate autoantigens of MS), and hardly reacted with either control or MS brain tissues.[24] This inconsistency indicates that autoantibody production within the CNS is probably not the initiating force in MS. However, there is no doubt the presence of OCB indicates abnormal B-cell activation within the CNS of patients with MS and suggests a role for B cells in the propagation of disease.

B CELLS AND DISEASE COURSE B-cell homeostasis and function within the CNS are relevant for the development and course of clinically definite MS (CDMS). In 85% of patients with MS the initial presentation is defined as a clinically isolated syndrome (CIS). Only about 60% of CIS cases will develop a second demyelinating event and therefore be diagnosed with CDMS over 20 years.[25] Identifying factors driving the fate of patients with CIS is relevant not only for a better prognosis and early intervention strategies but also for the understanding of the biological mechanisms behind the development of CDMS.

B-cell counts are increased in the CSF of patients with CIS and peripheral B-cell subsets show a markedly higher expression of the $\alpha 4$ subunit of the VLA-4 receptor, needed for their migration across the blood–brain barrier.[26] Abnormal B-cell activity is not only present at the first manifestation of the disease, but also predicts conversion to CDMS (table 1). CIS cases with OCB and polyspecific production of antibodies against measles, rubella, and varicella zoster viruses (the so-called "MRZ reaction") and high levels of the B-cell attractant chemokine CXCL13 in

Table 1 Studies testing B-cell activation markers as predicting factors of conversion from CIS to clinically definite MS

Predicting factor	Sample size	Mean follow-up time	Risk of conversion	Reference
OCB+	52 CIS	6 y	Increased risk: sensitivity = 91.42%; specificity = 94.11%	27
OCB+	415 CIS	50 mo	HR = 1.7; 95% CI = 1.1–2.7	28
OCB+	89 CIS	2 y	Increased risk: sensitivity = 96% (95% CI = 86–99.5); specificity = 33% (95% CI = 18.6–49.1)	31
MRZR+			Increased risk: sensitivity = 47% (95% CI = 32.5–61.7); specificity = 75% (95% CI = 58.8–87.3)	
OCB+ and MRI+	40 CIS	60 mo	RR = 9.1 (95% CI = 3.5–14.6)	29
OCB+	601 Optic neuritis	5.4 y	Increased risk: ROC = 0.89	30
High CSF CXCL13 levels	91 CIS	2 y	Increased risk: sensitivity = 62% (95% CI = 46–76); specificity = 76% (95% CI = 61–87)	32
High CSF CXCL13 levels	79 CIS	2 y	Increased risk: higher levels in converted patients ($p < 0.001$)	33
VH4/VH2 bias	10 CIS	2 y	Increased risk: VH4/VH2 bias more frequent in converted patients ($p = 0.03$)	36
Serum antimyelin antibodies	103 CIS	50.9 mo	Anti MOG + /anti MBP + HR = 76.5 (95% CI = 20.6–284.6)	37
			Anti MOG+/anti MBP − HR = 31 (95% CI = 9.5–104.5)	
Serum antimyelin antibodies	462 CIS	24 mo	IgG anti MOG + /anti MBP + HR = 1.04 (95% CI = 0.52–2.11)	38
			IgM anti MOG + /anti MBP + HR = 1.0 (95% CI = 0.63–1.57)	
			IgG anti MOG+/anti MBP − HR = 1.0 (95% CI = 0.63–1.6)	
			IgM anti MOG+/anti MBP − HR = 0.62 (95% CI = 0.41–0.94)	
			IgG anti MOG−/anti MBP + HR = 0.97 (95% CI = 0.58–1.6)	
			IgM anti MOG−/anti MBP + HR = 0.7 (95% CI = 0.39–1.27)	

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; HR = hazard ratio; IgG = immunoglobulin G; MRZR = MRZ reaction (production of antibodies against measles, rubella, and varicella zoster viruses); MS = multiple sclerosis; OCB = oligoclonal bands; RR = relative risk; ROC = area under receiver operator characteristic curve.

the CSF are at an increased risk of a second demyelinating event.[27–33] Other studies have also shown an overrepresentation of certain variable heavy chain sequences (VH4 and VH2 families) in B and plasma cells collected from CSF and plaques of patients with MS and that this feature may influence conversion to CDMS.[34–36]

The hypothesis that the presence of antibodies directed against myelin antigens in the serum of CIS cases could predict conversion to MS has also been investigated. While Berger et al.[37] reported that risk of conversion was greater in CIS cases with serum antibodies directed against MBP and MOG, these results have not been confirmed by a larger multicenter trial,[38] suggesting that anti-myelin antibody production is unlikely to mediate the role of B cells in CIS conversion.

Markers of B-cell presence and activation also correlate with disease activity and progression in CDMS (table 2). CSF CXCL13 levels are augmented in CIS, RRMS, secondary progressive MS (SPMS), and primary progressive MS (PPMS) and correlate with CSF total leukocyte and B-cell counts, intrathecal IgG synthesis, markers of demyelination and blood–brain barrier leakage, MRI activity, and relapse rate.[33,39,40] Serum levels of CXCL13 are also higher during periods of MRI activity.[41] Mature B-cell and plasma blast CSF counts correlate with MRI lesions.[42] Furthermore, disease progression is slower in OCB-negative (OCB−) than in OCB-positive (OCB+) patients.[43,44] Further evidence for a role of B cells in disease progression comes from pathologic studies showing the presence of lymphoid neogenesis in a substantial percentage of SPMS cases. Inflammatory aggregates organized as B-cell germinal center (GC)–like structures were observed in the subarachnoid space of SPMS cases and associated with features indicative of a more severe disease course.[45–47] Notably, a colocalization between these

Table 2 Studies investigating the role of B cells in disease activity and progression

B-cell marker	Sample size	Finding	Reference
CSF mature B and plasma cells	25 CIS and 20 RRMS	Higher in patients with Gd-enhancing lesions (mature B cells $p < 0.001$; plasma blasts $p < 0.001$)	42
		Higher in patients with >9 T2 lesions (mature B cells $p < 0.001$; plasma blasts $p = 0.009$)	
		Correlation with total CSF leukocyte count (mature B cells $\rho = 0.55$ $p < 0.001$; plasma blasts $\rho = 0.44$ $p < 0.001$)	
		Higher in patients with OCB (mature B cells $p < 0.001$; plasma blasts $p < 0.001$)	
		Correlation with CSF CXCL13 (mature B cells $\rho = 0.57$ $p < 0.001$; plasma blasts $\rho = 0.52$ $p < 0.001$)	
		Correlation with CSF matrix metalloproteinase 9 levels (mature B cells: $\rho = 0.415$ $p < 0.001$; plasma blasts: $\rho = 0.44$ $p < 0.001$)	
OCB	100 OCB+/100 OCB − MS	Time to DSS 4 significantly longer in OCB − than OCB+ (12.2 vs 7.2 years, respectively, HR = 0.6, 95% CI = 0.39–0.93)	43
		Time to DSS 6 significantly longer in OCB − than OCB+ (19.3 vs 10.9 years, respectively, HR = 0.5, 95% CI = 0.27–0.94)	
		Typical MRI features 36% less common in OCB − than OCB+ (95% CI = 24–48)	
OCB	1,404 OCB+/84 OCB − MS	OCB + patients have lower age at EDSS 6 (HR = 1.89; 95% CI = 1.19–2.99)	44
CSF CXCL13 levels	22 CIS and 58 RRMS	Direct correlation with number of Gd-enhancing lesions ($\rho = 0.46$; $p = 0.002$)	39
		Direct correlation with CSF MBP levels ($\rho = 0.44$; $p < 0.001$)	
		Direct correlation with total CSF leukocyte count ($\rho = 0.69$; $p < 0.001$)	
		Direct correlation with CSF B-cell count ($\rho = 0.83$; $p < 0.001$)	
		Direct correlation with intrathecal IgG synthesis ($\rho = 0.71$; $p < 0.001$)	
		Direct correlation with CSF matrix metalloproteinase 9 levels ($\rho = 0.83$; $p < 0.001$)	
	9 PPMS	Direct correlation with total CSF leukocyte count ($\rho = 0.68$; $p = 0.04$)	
	22 SPMS	Direct correlation with total CSF leukocyte count ($\rho = 0.46$; $p = 0.03$)	
CSF CXCL13 levels	323 RRMS	Higher levels in patients with higher relapse rate ($p < 0.001$) and higher number of MRI lesions ($p < 0.05$)	33
	323 RRMS, 40 SPMS, 24 PPMS	Direct correlation with total CSF leukocyte count ($\rho = 0.57$; $p < 0.0001$) and intrathecal IgG synthesis ($\rho = 0.47$; $p < 0.0001$)	
CSF CXCL13 levels	32 RRMS	Higher levels than in the control group but no difference between relapsing and nonrelapsing patients	40
Serum CXCL13 levels	74 RRMS	Direct correlation with MRI combined active lesions ($\rho = 0.24$, $p = 0.012$)	41
		Higher levels in patients with always active MRI than in those in complete ($p < 0.01$) or partial ($p = 0.01$) remission	
Meningeal GC-like structures	123 SPMS	Association with earlier age at onset, age at conversion to SPMS, age at wheelchair, and age at death ($p < 0.0001$)	47
		Association with higher total grey matter lesion area (6-fold increase, $p = 0.003$)	
Meningeal GC-like structures	37 SPMS	Association with pial to white matter gradient of neuronal loss	48

Abbreviations: CIS = clinically isolated syndrome; DSS = disability status scale; EDSS = Expanded Disability Status Scale; GC = germinal center; Gd = gadolinium; HR = hazard ratio; IgG = immunoglobulin G; OCB = oligoclonal bands; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

B-cell aggregates and subpial cortical lesions[46,47] and a gradient of neuronal loss in the cortical layers[48] were found, suggesting the release of soluble factors across the inflamed meninges may cause cortical damage.

B CELLS AND MS RISK FACTORS

The role of genetics. Complex disorders such as MS are conditions that have no single cause but result from a combination of genetic and environmental factors and their interactions.[49] It would be logical to hypothe-

size that these factors would have a direct influence on the cell type triggering the abnormal immune response in MS. This does not prove causation but it is an unconditional requirement for plausibility.

The main genetic locus in MS is located within the MHC class II region and corresponds to the *HLA-DRB1*1501* class II allele.[4] This allele has been found to increase the risk of MS in most populations studied and its presence in homozygosity increases the risk of MS more than 6-fold.[2,4]

A further stratification of patients based on the presence or absence of OCB has demonstrated that the *DRB1*1501* allele is strongly associated with the OCB+ subpopulation whereas the association tends to disappear in OCB− patients.[50–52] It has therefore been hypothesized that OCB− patients may represent a phenotypically similar but immunologically distinct entity.[50] However, OCB status can change during the course of MS,[53] perhaps suggesting that OCB negativity represents particular phases of the disease, or individuals with a weaker tendency to B-cell activation rather than a distinct condition. The *DRB1*1501* allele may be involved in this scenario, since MHC class II mediated presentation of antigens from B cells to CD4+ T cells is important for B-cell differentiation into GC B cells and plasma cells.[54]

Several non-MHC genes have been associated with MS susceptibility,[55–57] some of which, such as *CD40*, *CXCR4*, and *CXCR5*, appear particularly relevant for B-cell homeostasis and function. The transmembrane protein CD40 is constitutively present on B cells. Its cognate receptor CD154 is expressed in a wide range of cell types (mainly activated CD4+ and CD8+ T cells) and its binding with CD40 induces B-cell proliferation, chemokine and cytokine production, GC formation, differentiation into memory B and plasma cells and B-cell–mediated T-cell activation by upregulating MHC class II and costimulatory molecules expression.[58] In addition, the B-cell attractant CXCL13 acts through binding the receptor CXCR5, which is highly expressed on B cells and to a lesser extent T cells.[59] Together with *CXCR4*, these 2 genes are directly involved in GC development and their finely regulated expression in centroblasts and centrocytes is critical for correct GC organization.[60]

The role of environment. The environment also exerts a significant influence on MS susceptibility.[e1] A wealth of studies strongly support vitamin D deficiency as a key factor for MS. The prevalence of MS correlates with latitude and UV radiation[e2,e3] and both vitamin D intake and low vitamin D levels are inversely associated with risk of MS.[e4,e5] Possibly the strongest evidence for a role for vitamin D is the association of 2 genes involved in vitamin D biology with MS.[57]

As in other immune cell types, vitamin D influences development and functionality of B cells.[e6] This pleiotropic hormone plays an important role in B-cell homeostasis and function by decreasing cell proliferation, inducing apoptosis, and inhibiting plasma cell differentiation.[e7]

Using chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq) in B-cell lines, our group has shown the presence of 2,776 different vitamin D responsive elements (VDREs) through the entire genome bound by the vitamin D receptor (VDR). Genetic loci associated with MS are strikingly enriched for VDR binding sites. In other words, vitamin D regulates the expression of genes which are associated with MS susceptibility in B cells including *HLA-DRB1*, *CD40*, *CXCR4*, and *CXCR5*.[e8]

The Epstein-Barr virus (EBV) may also be involved in the pathophysiology of MS. Although a great majority (more than 90%) of the general population appears to encounter EBV at some point during life, nearly all patients with MS (>99.5%) have been infected with EBV.[2] The risk of MS is increased in individuals with either high anti-EBV antibody titers or a history of infectious mononucleosis (IM).[2e9] These observations do not arise from a shared genetic susceptibility since the *HLA-DRB1*1501* class II allele is not associated with IM.[e10]

This is relevant as EBV is a DNA human γ herpesvirus which primarily infects B cells and is able to immortalize them in vitro and induce lymphoproliferative disorders in vivo.[e11] Different proteins encoded by EBV, in particular members of the Epstein-Barr nuclear antigen (EBNA) and latent membrane protein (LMP) families, influence the expression of a number of genes involved in cell adhesion or signaling, transcription, RNA processing, immune processes, and cell-cycle regulation.[e11-e14]

Interestingly, a common thread seems to link EBV and vitamin D B-cell gene regulation pathways. One way EBV influences gene expression is by an EBNA-3 mediated blockage of the VDR.[e15] As an exploratory analysis, we used the expression profiles of lymphoblastoid cell lines (LCLs) obtained by infecting primary B cells with an EBV mutant strain lacking the EBNA-3 gene[e13] and our VDR ChIP-Seq map[e8] to explore to what extent EBNA-3 may influence the expression of vitamin D–responsive genes. Almost 30% of the genes which are regulated by EBNA-3 are characterized by the presence of a VDRE, which is much greater than expected by chance ($p = 0.003$). In a gene ontology

analysis we found that these genes with both an EBNA-3 and VDR influence are involved in cell proliferation, apoptosis, and immune response.

Other findings supporting a link between B cells, EBV, and MS come from pathologic studies reporting the presence of markers of latent EBV infection in a very high percentage of brain-infiltrating B and plasma cells in nearly all MS samples examined. The persistence of EBV was particularly enriched in meningeal B-cell follicles where viral reactivation (as defined by the presence of the early lytic protein BFRF1) was also observed.[e16] By using laser microdissection and preamplifying EBV transcripts, subsequent experiments significantly increased the sensitivity of EBV detection.[e17] EBV-positive B cells were also found to express the B-cell activating factor, which previous studies had shown to be upregulated by EBV proteins in B-cell lines and overexpressed in MS brain.[e17-e19]

However, regardless of how attractive the underlying biological rationale of the EBV presence in the CNS of patients with MS is, caution is needed since other groups have not been able to replicate these findings.[e20-e25] For example, Sargsyan et al.[e25] did not detect any EBV transcript in MS CSF B and plasma cells or in most of the actively demyelinating MS plaques analyzed. A single EBV-specific transcript (EBER-1) was found in a subset of MS plaques previously shown to be EBV DNA positive indicating the absence of EBV reactivation or abnormal latency programs.[e25] Differences in the ascertainment of cases, sample preparation, and methods may be possible confounding factors,[e20,e26] but replication is still needed to claim the presence and activation of EBV in MS brain as consistent features of the disease.

Another observation that needs replication is decreased CD8+ T-cell reactivity to autologous EBV-infected lymphoblastoid cell lines in patients with MS compared with healthy subjects.[e27] This may predispose to the development of MS by allowing the accumulation of EBV-infected B cells in the MS brain. However, these data are contrary to other findings of higher EBV-specific CD8+ T-cell response in the blood of patients with CIS and normal response in patients with MS.[e28]

B-CELL DEPLETION IN MS: INSIGHT INTO BIOLOGICAL MECHANISMS The development of monoclonal antibodies has represented one of the most significant changes in MS therapy. To date, several different molecules have been tested and the therapeutic efficacy of many of them promises to be much greater than currently available treatments.[e29] Although the antigens targeted by such molecules are known, the identification of the mechanism underlying their therapeutic action is unclear since generally these antibodies target a wide range of immune cells.

For example, the monoclonal antibody natalizumab acts by blocking the $\alpha 4$ subunit of the VLA-4 receptor, which is needed for most leukocyte migration across the blood–brain barrier. Similarly, the impressively effective alemtuzumab causes complete depletion of all cells expressing the CD52 molecule which comprises T, B, natural killer and dendritic cells, most monocytes, and macrophages.[e29]

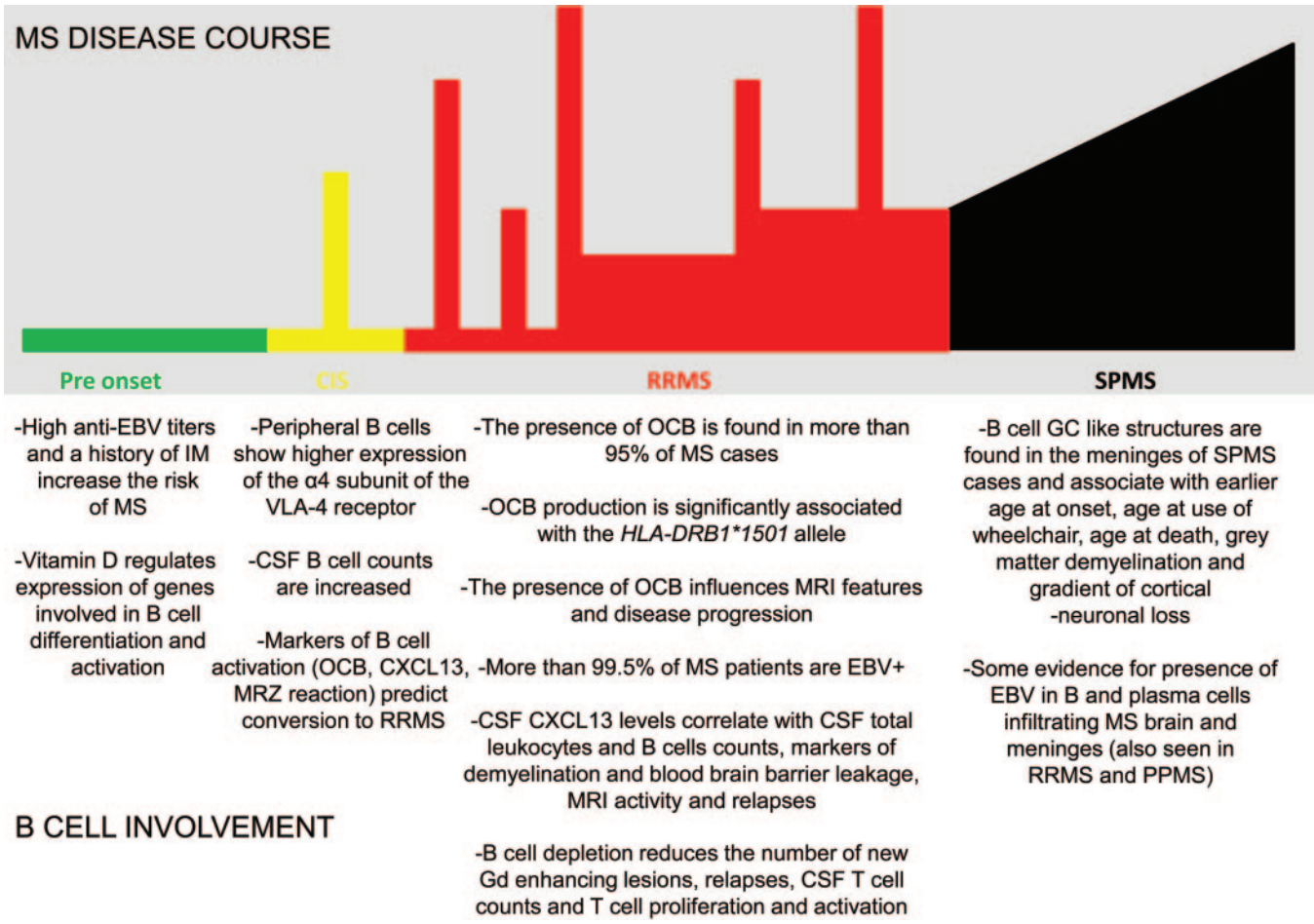
Interestingly, the only cell-specific antibody which has proved to be highly efficient in RRMS, rituximab, acts on the B-cell compartment, particularly by depleting the B cells positive for the CD20 antigen.[e30] This transmembrane protein is expressed in different stages of B-cell differentiation, from pre-B cells to naive and memory B cells, but is absent in earlier stages (pro-B) and plasma cells.[e30,e31] Clinical trials in RRMS have shown that rituximab causes a nearly complete depletion of CD20+ B cells and reduces the number of new Gd-enhancing lesions and the proportion of patients experiencing relapses.[e32-e34] Similar results have been obtained in trials testing the second generation of anti-CD20 molecules (ocrelizumab and ofatumumab).[e30]

The fact that treatments specifically targeting B cells are effective in RRMS provides not only further support for the pathogenic role of B cells in MS but also the opportunity to elucidate how B cells may be acting. Notably, anti CD20 treatment does not target plasma cells and the influence on CSF IgG levels and OCB is minimal or absent.[e35,e36] Therefore, it is unlikely that autoantibody production mediates the pathogenic role of B cells in MS.

Rather than producing autoantibodies, B cells may play a central role in influencing the T-cell response in patients with MS. As described above, B cells can function as professional antigen-presenting cells able to initiate T-cell–specific responses.[e37,e38] Studies have suggested that a proportion of peripheral B cells in patients with RRMS can elicit CD4+ T-cell activation and proliferation in response to myelin antigens via direct antigen presentation.[e39] However, B cells could also influence the T-cell response through cytokine and chemokine production (bystander mechanisms). These hypotheses find support in both animal and human studies of B-cell depletion.

Interestingly, the effects of B cell depletion on EAE models range from EAE prevention to exacerbation depending on the timing of anti-CD20 treatment and the peptide used to induce CNS inflammation.[e40,e41] These findings result from B cells having either regulatory or proinflammatory phenotypes and influencing

Figure B-cell involvement in different phases of multiple sclerosis (MS)



Environmental risk factors influence B cells prior to disease onset; B-cell abnormalities within the CNS are already present at time of clinically isolated syndrome (CIS) diagnosis and predict conversion to clinically definite MS; during relapsing-remitting MS (RRMS), markers of B-cell activation correlate with disease course and MRI activity while B-cell depletion significantly reduces MRI lesions and onset of relapses; organized B-cell structures are found in the meningeal space of patients with secondary progressive MS (SPMS) and their presence is associated with a worse outcome. EBV = Epstein-Barr virus; GC = germinal center; IM = infectious mononucleosis; OCB = oligoclonal bands.

the T-cell response through cytokine production and antigen presentation.[e40,e41]

Similarly to EAE, in human MS rituximab treatment influences the interplay between B and T cells. Studies have shown that rituximab administration reduces CSF counts of both B cells and T cells (95% and 50% mean reductions, respectively).[e35,e42] This decrease in T-cell counts correlated with a reduction of CXCL13 levels between pre and post treatment CSF paired samples.[e42] Since CXCR5 (the receptor for CXCL13) is found on B cells and ~20% of CD4+ T cells in blood and CSF of patients with MS[59] and B cells can promote maturation of follicular dendritic cells and CXCL13 production in positive feedback fashion,[e43] the authors speculated that lack of B cells may decrease the number of T cells within the CNS by reducing chemokine secretion.[e42]

Another hypothesis is that B-cell depletion may impair T-cell activation and trafficking across the blood–brain barrier. In line with this, Bar Or et al. have shown that CD4+ and CD8+ T cells from patients with MS exhibited reduced proliferation and IFN-γ and IL-17 production after B cell depletion as compared to pretreatment levels. Notably, these effects could be partially reversed by adding the supernatant of activated B-cell cultures obtained from untreated patients, implicating the presence of soluble factors able to activate T cells. Further experiments identified the B-cell–produced molecules lymphotoxin and tumor necrosis factor–α as relevant in mediating this mechanism.[e44]

Although these observations indicate that T cells may be partly mediating the pathogenic role of B cells in MS, the secretion of soluble factors other than antibodies from B cells, located in either meninges or brain parenchyma, may directly induce inflammation and consequent demyelination. Notably, these hypotheses are not mutually

Table 3 Hill's criteria for causation fulfilled by current knowledge on B cells in MS

Hill's criteria	Definition	Fulfilled by B cells?
Strength	The association between the causing and consequent events should be strong	Yes: OCB are found in more than 95% of patients with MS
Consistency	Findings must be consistent and reproducible	Yes: Observations are consistent between different studies and investigators
Specificity	A single putative cause produces a specific effect	No: B cells are involved in other diseases
Temporality	The causing factor must precede the consequence	Yes: B-cell abnormalities are present from the beginning of the disease
Biological gradient	A dose response curve between cause and effect	Yes: B-cell abnormalities are associated with conversion to MS, disease course, and progression
Plausibility	The causation suspected should be biologically plausible	Yes: MS risk factors influence B-cell proliferation and function and B cells are involved in other immune disorders
Coherence	The interpretation of the data should not seriously conflict with the natural history and biology of the disease	Yes: A causative role for B cells does not conflict with either history or biology of MS
Experiment	The condition can be altered by an appropriate experimental regimen	Yes: B-cell depletion significantly impacts MRI features and onset of relapses
Analogy	The effect of similar factors should be considered	Yes: A causative role for B cells does not exclude a role for other cell types

Abbreviations: MS = multiple sclerosis; OCB = oligoclonal bands.

exclusive and multiple mechanisms are likely to take place simultaneously.

DISCUSSION Establishing a causal relationship is not easy and requires a number of observations. In 1965, Sir Austin Bradford Hill[e45] outlined a number of criteria with the intent to define "what aspects of that association should we especially consider before deciding that the most likely interpretation of it is causation."

As Hill wanted to clarify, "none of these 9 viewpoints can bring indisputable evidence for or against the cause and effect hypothesis and none can be required as a sine qua non." However, it is interesting to see how many and how well these criteria are met by B cells in MS today (figure and table 3). Strikingly, B cells satisfy 8 out of the 9 features of causation and in particular fulfill the main requirements that B-cell abnormalities are strongly associated with MS, are influenced by MS risk factors, precede its clinical onset, influence disease course, and can be modified by a specific intervention strategy.

Current studies unequivocally indicate that the general view of MS as a condition mediated only by T cells is overly simplistic and that B cells influence the pathogenesis of MS. Although the understanding of MS causation will require the unification of all the pieces of a complex immunologic puzzle, particularly with regard to B–T cell interactions, these observations suggest that further elucidation of the role of B cells in MS will lead to the development of novel disease treatment and prevention strategies.

AUTHOR CONTRIBUTIONS

Study concept and design: Drs. Disanto and Ramagopalan. Drafting of the manuscript: Dr. Disanto. Critical revision of the manuscript for important intellectual content: Drs. Morahan, Barnett, Giovannoni, and Ramagopalan.

DISCLOSURE

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REFERENCES

1. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000;343:938–952.
2. Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. Lancet Neurol 2010;9:727–739.
3. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. Brain Pathol 2007;17:210–218.
4. Ramagopalan SV, Ebers GC. Multiple sclerosis: major histocompatibility complexity and antigen presentation. Genome Med 2009;1:105.
5. Klein L, Hinterberger M, Wirnsberger G, Kyewski B. Antigen presentation in the thymus for positive selection and central tolerance induction. Nat Rev Immunol 2009;9:833–844.
6. Kasper LH, Shoemaker J. Multiple sclerosis immunology: the healthy immune system vs the MS immune system. Neurology 2010;74(suppl 1):S2–S8.
7. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 2004;55:458–468.
8. Henderson AP, Barnett MH, Parratt JD, Prineas JW. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol 2009;66:739–753.
9. Babbe H, Roers A, Waisman A, et al. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation

and single cell polymerase chain reaction. J Exp Med 2000; 192:393–404.
10. Gay FW, Drye TJ, Dick GW, Esiri MM. The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of the primary demyelinating lesion. Brain 1997;120: 1461–1483.
11. Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol 2008;7:796–804.
12. van Oosten BW, Lai M, Hodgkinson S, et al. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial Neurology 1997;49:351–357.
13. Brok HP, van Meurs M, Blezer E, et al. Prevention of experimental autoimmune encephalomyelitis in common marmosets using an anti-IL-12p40 monoclonal antibody. J Immunol 2002;169:6554–6563.
14. Waldor MK, Sriram S, Hardy R, et al. Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. Science 1985;227: 415–417.
15. Handel AE, Lincoln MR, Ramagopalan SV. Of mice and men: experimental autoimmune encephalitis and multiple sclerosis. Eur J Clin Invest Epub 2011.
16. Freedman MS, Thompson EJ, Deisenhammer F, et al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch Neurol 2005;62:865–870.
17. Owens GP, Ritchie AM, Burgoon MP, Williamson RA, Corboy JR, Gilden DH. Single-cell repertoire analysis demonstrates that clonal expansion is a prominent feature of the B cell response in multiple sclerosis cerebrospinal fluid. J Immunol 2003;171:2725–2733.
18. Lovato L, Willis SN, Rodig SJ, et al. Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. Brain 2011;134:534–541.
19. Obermeier B, Lovato L, Mentele R, et al. Related B cell clones that populate the CSF and CNS of patients with multiple sclerosis produce CSF immunoglobulin. J Neuroimmunol 2011;233:245–248.
20. Prineas JW. Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. Science 1979;203:1123–1125.
21. Breij EC, Brink BP, Veerhuis R, et al. Homogeneity of active demyelinating lesions in established multiple sclerosis. Ann Neurol 2008;63:16–25.
22. Weber MS, Hemmer B, Cepok S. The role of antibodies in multiple sclerosis. Biochim Biophys Acta 2011;1812:239–245.
23. von Budingen HC, Harrer MD, Kuenzle S, Meier M, Goebels N. Clonally expanded plasma cells in the cerebrospinal fluid of MS patients produce myelin-specific antibodies. Eur J Immunol 2008;38:2014–2023.
24. Owens GP, Bennett JL, Lassmann H, et al. Antibodies produced by clonally expanded plasma cells in multiple sclerosis cerebrospinal fluid. Ann Neurol 2009;65:639–649.
25. Fisniku LK, Brex PA, Altmann DR, et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain 2008;131:808–817.
26. Lee-Chang C, Zephir H, Top I, et al. B-cell subsets upregulate alpha4 integrin and accumulate in the cerebrospinal fluid in clinically isolated syndrome suggestive of multiple sclerosis onset. Neurosci Lett 2011;487:273–277.
27. Masjuan J, Alvarez-Cermeno JC, Garcia-Barragan N, et al. Clinically isolated syndromes: a new oligoclonal band test accurately predicts conversion to MS. Neurology 2006;66: 576–578.
28. Tintore M, Rovira A, Rio J, et al. Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis? Neurology 2008;70:1079–1083.
29. Ignacio RJ, Liliana P, Edgardo C. Oligoclonal bands and MRI in clinically isolated syndromes: predicting conversion time to multiple sclerosis. J Neurol 2010;257:1188–1191.
30. Skov AG, Skov T, Frederiksen JL. Oligoclonal bands predict multiple sclerosis after optic neuritis: a literature survey. Mult Scler 2011;17:404–410.
31. Brettschneider J, Tumani H, Kiechle U, et al. IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome. PLoS One 2009;4:e7638.
32. Brettschneider J, Czerwoniak A, Senel M, et al. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). PLoS One 2010;5:e11986.
33. Khademi M, Kockum I, Andersson ML, et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler 2011;17: 335–343.
34. Owens GP, Kraus H, Burgoon MP, Smith-Jensen T, Devlin ME, Gilden DH. Restricted use of VH4 germline segments in an acute multiple sclerosis brain. Ann Neurol 1998;43:236–243.
35. Owens GP, Winges KM, Ritchie AM, et al. VH4 gene segments dominate the intrathecal humoral immune response in multiple sclerosis. J Immunol 2007;179:6343–6351.
36. Bennett JL, Haubold K, Ritchie AM, et al. CSF IgG heavy-chain bias in patients at the time of a clinically isolated syndrome. J Neuroimmunol 2008;199:126–132.
37. Berger T, Rubner P, Schautzer F, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med 2003;349: 139–145.
38. Kuhle J, Pohl C, Mehling M, et al. Lack of association between antimyelin antibodies and progression to multiple sclerosis. N Engl J Med 2007;356:371–378.
39. Sellebjerg F, Bornsen L, Khademi M, et al. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. Neurology 2009;73:2003–2010.
40. Kalinowska-Lyszczarz A, Szczucinski A, Pawlak MA, Losy J. Clinical study on CXCL13, CCL17, CCL20 and IL-17 as immune cell migration navigators in relapsing-remitting multiple sclerosis patients J Neurol Sci 2011;300:81–85.
41. Festa ED, Hankiewicz K, Kim S, et al. Serum levels of CXCL13 are elevated in active multiple sclerosis. Mult Scler 2009;15:1271–1279.
42. Kuenz B, Lutterotti A, Ehling R, et al. Cerebrospinal fluid B cells correlate with early brain inflammation in multiple sclerosis. PLoS One 2008;3:e2559.
43. Joseph FG, Hirst CL, Pickersgill TP, Ben-Shlomo Y, Robertson NP, Scolding NJ. CSF oligoclonal band status informs prognosis in multiple sclerosis: a case control study

of 100 patients. J Neurol Neurosurg Psychiatry 2009;80:292–296.
44. Imrell K, Greiner E, Hillert J, Masterman T. HLA-DRB115 and cerebrospinal-fluid-specific oligoclonal immunoglobulin G bands lower age at attainment of important disease milestones in multiple sclerosis. J Neuroimmunol 2009;210:128–130.
45. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 2004;14:164–174.
46. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089–1104.
47. Howell OW, Reeves CA, Nicholas R, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain Epub 2011.
48. Magliozzi R, Howell OW, Reeves C, et al. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol 2010;68:477–493.
49. Dempfle A, Scherag A, Hein R, Beckmann L, Chang-Claude J, Schafer H. Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. Eur J Hum Genet 2008;16:1164–1172.
50. Imrell K, Landtblom AM, Hillert J, Masterman T. Multiple sclerosis with and without CSF bands: clinically indistinguishable but immunogenetically distinct. Neurology 2006;67:1062–1064.
51. Romero-Pinel L, Martinez-Yelamos S, Bau L, et al. Association of HLA-DRB1*15 allele and CSF oligoclonal bands in a Spanish multiple sclerosis cohort. Eur J Neurol 2011.
52. Wu JS, Qiu W, Castley A, et al. Presence of CSF oligoclonal bands (OCB) is associated with the HLA-DRB1 genotype in a West Australian multiple sclerosis cohort. J Neurol Sci 2010;288:63–67.
53. Zeman AZ, Kidd D, McLean BN, et al. A study of oligoclonal band negative multiple sclerosis. J Neurol Neurosurg Psychiatry 1996;60:27–30.
54. Shimoda M, Li T, Pihkala JP, Koni PA. Role of MHC class II on memory B cells in post-germinal center B cell homeostasis and memory response. J Immunol 2006;176:2122–2133.
55. ANZgene. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. Nat Genet 2009;41:824–828.
56. De Jager PL, Jia X, Wang J, et al. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet 2009;41:776–782.
57. Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476:214–219.
58. Graham JP, Arcipowski KM, Bishop GA. Differential B-lymphocyte regulation by CD40 and its viral mimic, latent membrane protein 1. Immunol Rev 2010;237:226–248.
59. Krumbholz M, Theil D, Cepok S, et al. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain 2006;129:200–211.
60. Allen CD, Ansel KM, Low C, et al. Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. Nat Immunol 2004;5:943–952.